The Biochemical Confirmation of \textit{Salmonella} and \textit{Listeria} Pathogen Microorganisms from Food with the Help of \textit{Vidas} and \textit{Vitek} Rapid Analyzers

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\textbf{Abstract.} \textit{Listeria monocytogenes} is widely spread in the environment and represents a potential risk when non-processed, partially processed of fermented food is consumed.

The \textit{VIDAS} \textit{L. monocytogenes} II test is a rapid screening test and represents an alternative to the long-term alternative identification. This test can be used for the direct screening of \textit{L. monocytogenes} in the food samples and the environment.

\textit{Salmonella} is one of the main causes of food toxic infection. \textit{Salmonella} organisms are ubiquitous in the environment and reach the intestine of humans and other mammals, especially domestic and predominately (rodents), birds, reptiles and even insects. (Ancuța M. Rotar, S. Apostu, 2009).

Detecting \textit{Salmonella} using classical methods: preenrichment steps, enrichment, isolation and confirmation can last up to 5 days. The screening techniques, based on the immunoenzymatic analysis (IEA) offering the possibility of simplifying and the accelerating the detection.

This way, the automatic IEA \textit{Salmonella} \textit{VIDAS} test (SLM) for detecting \textit{Salmonella} in food samples and environment uses a mixture of capture antibodies with high specificity both against O antigens and H antigens, which allows the detection of both mobile and immobile \textit{Salmonella}.

The \textit{VITEK} 2 GP and GN cards were developed to satisfy the growing demand for quality control measures within the food industry and provide identification of organisms in just hours, while traditional methods take days to deliver results. The GN card provides automatic identification of the most significant fermenting and non-fermenting Gram -negative bacilli, including several \textit{Salmonella} species and \textit{E. coli} O157, in addition to the select agent organisms \textit{Brucella melitensis}, \textit{Francisella tularensis}, \textit{Burkholderia mallei}, \textit{Burkholderia pseudomallei} and \textit{Yersinia pestis}. The \textit{VITEK} 2 GP card provides rapid identification of common Gram -positive organisms, including \textit{Listeria} and \textit{Staphylococcus} species (bioMérieux Industry).

\textbf{Keywords:} \textit{Vidas}, \textit{Vitek}, food, pathogen microorganisms, biochemical confirmation.

\textbf{INTRODUCTION}

“Rapid and accurate identification of foodborne pathogens and biological threat organisms is crucial for ensuring food safety and protecting consumer health,” stated Alexandre Mérieux, bioMérieux Corporate Vice.

The usual classification of \textit{Listeria} genus includes 6 species of short mobile bacilli, Gram-positive and asporous. These organisms are the catalase +, oxidase -, they hydrolyse the esculin and ferment the glucose without gas production. They can develop on a large scale of temperature and pH and can stand high concentrations of sodium chloride.

\textit{Listeria monocytogenes} is the only species considered pathogenic for humans. Listeriosis in humans may cause pathologies such as meningitis, septicaemia, encephalitis and abortions. Groups at risk include pregnant women, neonates, immune-compromised patients,
and the elderly. Members of the genus *Listeria* are ubiquitous and only one species is pathogenic. *Listeria* has been isolated from various food products including dairy products, meat, vegetables, and seafood, as well as from environmental samples taken, in particular, from food processing plants.

*Salmonella* is one of the main causes of food poisoning in the world. Symptoms due to a *Salmonella* infection are: fever, diarrhea and abdominal cramps. *Salmonella* can be found in the intestinal tracts of humans and some animals, including birds. The transmission to human is usually caused by eating foods contaminated with animal faeces. (bioMérieux Industry).

A new innovation was early introduced with the manual api identification strip and continues with the automated advanced colorimetric identification, the VITEK 2 Compact, launched in 2005.

**MATERIALS AND METHODS**

**The work procedure for VIDAS *L. monocytogenes* and VIDAS *Salmonella***

VIDAS *L. monocytogenes* II is an immunoenzymatic fluorescent test (ELFA) used on the VIDAS automatic instrument for the specific detection of *Listeria monocytogenes*.

The Solid Phase Recipient (SPR) serves both as a solid phase and pipetting device. The inside of SPR is covered with anti-*L monocytogenes* antibodies which are absorbed on its surface. The reagents for the analysis are ready to use and pre-disposed on the strips sealed with reagents.

All the steps of analysis are followed automatically by the instrument. The reaction medium is circulated inside and outside SPR several times.

The sample is put on the strip with reagents. The present antigens will attach to the anti-*Listeria monocytogenes* antibodies that cover the interior of SPR.

The untapped components of the sample are washed away.

The monoclonal antibodies marked with the alkaline phosphatase are circulated inside and outside of SPR and connected to any *L. monocytogenes* antigen that, in their turn are connected to the antibodies from the SPR wall. The next washing steps clear the unbound conjugate.

During the final step of detection, the underlayer (4-Methyl-umbelliferyl phosphate) is circulated inside and outside SPR. The conjugate enzyme catalyze the hydrolysis of this underlayer in a fluorescent product (4-Methyl-umbelliferone), its fluorescence being measured at 450 nm.

The strip is formed of 10 wells covered with sealed labeled foil. The label contains a barcode that indicates mainly the code of analysis, the kit batch number and the expiring date. The foil of the first well is punctured for facilitating the insertion of the sample. The last well of each strip is an impoundment in which the fluorometric measurement is done. The wells from the central section of the strip contain different reagents that are necessary for the test.

The work procedure follows the same steps as in the case of VIDAS *Salmonella*, but the inside of SPR is covered with anti-*Salmonella* antibodies that are absorbed on its surface.

**The official N°2004.02 method (protocol for VIDAS® LMO2)**

All types of food

- Aseptically, it is added 25g (or 25ml) from the 225ml sample of Half-FRASER broth.
- Homogenization using a Stomacher® bag.
• Incubating for de 25 ± 1 h at 30 ± 1°C.
• After incubation, it is mixed and 1ml of suspension is transferred in 10 ml of FRASER broth.
• Incubation for 25 ± 1 h at 30 ± 1°C.
• 500 µl of the obtained suspension are dropped in the sample well.
• The SPR®s and strips are introduced in the instrument, it is checked whether the colored labels, containing the analysis code from the SPR, match with the Reagent Strips.
• The analysis is initialized according to the instructions from the Operator’s Manual. All the analysis steps are followed automatically by the instrument. The results are obtained in approximately 70 minutes.

The VIDAS și VYTEK rapid tests are used only when a certain microorganism is suspected to be present in the analysed sample, using classical methods.


1. **Pre-enrichment**
   • Aseptically, 25g (or 25 ml) of sample are added in the peptonate tamponade water or a volume of the sample for nine volumes of a pre-enrichment broth is homogenized in a Stomacher bag.
   • Incubation for de 18- 24 h at 35 ± 1°C.

2. **Enrichement**
   • After incubation 1 ml of suspension is trasferred into 10 ml of Tetrathionate broth.
   • Incubation for 6-8 h at 41-42°C (with the exception of unprocessed food of food having a rich microbial content).
   • This is paralleled by the transfer of 0.1 ml of pre-enrichment broth into 10 ml of Rappaport Vassiliadios broth (RV). Incubation for 6-8 h at 41-42°C (with the exception of unprocessed food of food having a rich microbial content). In the case of unprocessed food of food having a rich microbial content the selective broths are incubated for 18-24 h.

3. **Post-enrichment**
   • After incubation, 1 ml of RV broth in 10ml of M broth.
   • In another tube of M broth, 1ml of Tetrathionate broth is transferred.
   • The M broths are incubated for 18-24 h at 41-42°C (unprocessed food or food having a rich microbial content).
   • For the unprocessed food or food having a rich microbial content, the M broths are incubated for 6-8 h at 41-42°C.
   • The re-incubation of the selective enrichment broths at 41-42°C for a total incubation time of 24 ± 2 h - used for the confirmation of the VIDAS® pozitive results.

   • After incubation, both broths are homogenized. After the water bathing is used, 1 ml from each broth is transferred in a single tube. The tube is sealed. It is heated for 15 ± 1 minute at 95-100°C, then the tube is cooled, the previously heated broth is homogenized and 0,5 ml of it are transferred, from the VIDAS strip, in the sample well.
• If the VIDAS® Heat and Go is used, 0.25 ml of each broth is transferred into the sample well from the strip. It is heated for 15 ± 1 minutes according to the VIDAS Heat and Go User’s Manual. The strip is taken out and cooled for 10 minutes.
• The VIDAS test is initialized.

The work procedure for VITEK L. monocytogenes and VIDAS Salmonella

The VITEK 2 Gram-Positive (GP) card is certified for the identification of *Listeria* and *Staphylococcus* species and the VITEK 2 Gram-Negative (GN) card is certified to identify Gram-negative organisms such as *Salmonella* and *E. coli O157*. (bioMérieux Industry).

Firstly, a pure bacterial culture is obtained, of 24 hours (it is started from a single colony), the inoculum is prepared – in 3 ml of sterile serum, the bacterial suspension is standardized with DENSICHEK between 0.5 – 0.63 McFarland.

From the obtained suspensions, different volumes are transferred into the cards for the identification of bacterial species (the test cards are made of plastic and contain 64 microcells, each cell containing a specific biochemical dehydrated underlayer).

- for GN AST – the Gram-negative bacilli - 145 µl in a 3 ml sterile serum tube.
- for GP AST – 280 µl of Gram-positive cocci are transferred into a tube with 3 ml of sterile serum.

The card inoculation is automatically made, the distribution time is 70 seconds for a 10 card cassette; after sealing, the cards are introduced in the identification system and read.

The card reading is based on establishing the biochemical methods and on the new developed underlayers that measure the use of the carbon source, the enzymatic activities and resistance.

The interpretation of the antibiogram results is based on the S,I,R criteria, according to the international standards (NCCLS).

RESULTS AND DISCUSSION

Using the two types of rapid analyzers - VIDAS and VITEK -, in the first half of 2010, a total number of 2821 samples for *Salmonella* and 383 samples for *Listeria monocytogenes* were analyzed at the Health Veterinary Department in Cluj-Napoca.

From the total of analyzed samples for *Listeria monocytogenes* 372 results were negative and only one was positive.

The analysis was made on samples from all food categories – milk and dairy products, beef, chicken, cooked and semi-processed food, fish products, confectionery and bakery products, that came from stores from Cluj county.

The presence of *Listeria monocytogenes* was noticed on a sample from fish fillet.

From the total of analyzed samples for *Salmonella* Group B and C, has resulted 2792 negative and 27 positive.

The types of analyzed sample for *Listeria monocytogenes*

<table>
<thead>
<tr>
<th>Crt. no.</th>
<th>Sample</th>
<th>No. of analyzed samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Unpasteurized cow’s milk cheese</td>
<td>41</td>
</tr>
<tr>
<td>2.</td>
<td>Unpasteurized sheep’s milk cheese</td>
<td>7</td>
</tr>
<tr>
<td>3.</td>
<td>Unpasteurized goat’s milk cheese</td>
<td>1</td>
</tr>
<tr>
<td>4.</td>
<td>Sour cream</td>
<td>2</td>
</tr>
</tbody>
</table>

Tab. 1
Fig. 1. Results of the *Salmonella* strains identification with the help of the VIDAS, VITEK 2 Compact automatic analyzers

After the analysis, the presence of *Salmonella* was noticed in 27 samples, the presence of *Salmonella* Group C was observed at 15 of them, and in the other 12, the presence of *Salmonella* group B serotypes.

Samples that confirmed the presence of *Salmonella* species

<table>
<thead>
<tr>
<th>Crt. No.</th>
<th>Sample type</th>
<th>No. of positive samples</th>
<th>The serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pressed cheese</td>
<td>1</td>
<td><em>Salmonella enteritidis</em></td>
</tr>
<tr>
<td>2</td>
<td>Sack cheese</td>
<td>1</td>
<td><em>Salmonella enteritidis</em></td>
</tr>
<tr>
<td>3</td>
<td>Tenderloin</td>
<td>1</td>
<td><em>Salmonella enteritidis</em></td>
</tr>
<tr>
<td>4</td>
<td>Frozen drumsticks</td>
<td>1</td>
<td><em>Salmonella infantis</em></td>
</tr>
<tr>
<td>5</td>
<td>Chicken neck skin</td>
<td>6</td>
<td><em>Salmonella infantis</em></td>
</tr>
<tr>
<td>6</td>
<td>Mixture of minced meat</td>
<td>1</td>
<td><em>Salmonella derby</em></td>
</tr>
<tr>
<td>7</td>
<td>Pork sausages</td>
<td>1</td>
<td><em>Salmonella derby</em></td>
</tr>
<tr>
<td>8</td>
<td>Minced beef</td>
<td>2</td>
<td><em>Salmonella typhimurium</em></td>
</tr>
<tr>
<td>9</td>
<td>Minced meat</td>
<td>1</td>
<td><em>Salmonella typhimurium</em></td>
</tr>
<tr>
<td>10</td>
<td>Fresh sausages</td>
<td>1</td>
<td><em>Salmonella typhimurium</em></td>
</tr>
<tr>
<td>11</td>
<td>Chicken liver</td>
<td>2</td>
<td><em>Salmonella infantis</em></td>
</tr>
<tr>
<td>12</td>
<td>Chicken gizzard</td>
<td>2</td>
<td><em>Salmonella infantis</em></td>
</tr>
<tr>
<td>13</td>
<td>Minced meat for meat balls</td>
<td>1</td>
<td><em>Salmonella typhimurium</em></td>
</tr>
<tr>
<td>14</td>
<td>Minced calf</td>
<td>1</td>
<td><em>Salmonella typhimurium</em></td>
</tr>
<tr>
<td>15</td>
<td>Rustic sausages</td>
<td>1</td>
<td><em>Salmonella typhimurium</em></td>
</tr>
<tr>
<td>16</td>
<td>Fowl meat balls</td>
<td>1</td>
<td><em>Salmonella infantis</em></td>
</tr>
<tr>
<td>17</td>
<td>Refrigerated fowl sausages</td>
<td>1</td>
<td><em>Salmonella infantis</em></td>
</tr>
<tr>
<td>18</td>
<td>Minced fowl</td>
<td>1</td>
<td><em>Salmonella infantis</em></td>
</tr>
</tbody>
</table>
CONCLUSIONS

The purpose of this study was that of investigating, through rapid detection methods, the degree of contamination with pathogen microorganisms of the *Listeria* and *Salmonella* type. The investigations confirmed through the rapid methods described above a number of 27 positive samples for *Salmonella* and a positive sample for *Listeria*.

*Salmonella* are organisms that are ubiquitous in the environment, coming from the human and other mammals’ intestine. The human factor as a primary source can contaminate food and feed. It intervenes decisively through incorrect manipulation, transport, storing and processing activities. Growing domestic animals intensively (especially poultry), the industrialization of milk and fish processing on a wide scale, recycling the fish waste (resulted from this industry for making feed) favored the continuous dominance of this pathogen in the food chain. (Ancuța M. Rotar, S. Apostu, 2009).

In nature there are many *L. monocytogenes* reservoirs: the environment, the animals, the humans and other living beings. From the environment, listeriae are frequently isolated from soil, water, decomposing plants. The extended listeriae reservoir is represented by almost all beings and their environment, this bacteria being ubiquitous. (Ancuța M. Rotar, S. Apostu, 2009).

We can conclude that through the presence in food of the two types of pathogen microorganisms - *Salmonella* and *Listeria* - there is a real danger of food toxic infection. Thus, the measures of control through rapid diagnosis and the prevention are meant to provide security to the consumer.

REFERENCES

8. ***www.biomerieux-industry.com***